UNIVERSITY OF THESSALY

SCHOOL OF MEDICINE

LABORATORY OF BIOMATHEMATICS

M.SC. "RESEARCH METHODOLOGY IN BIOMEDICINE, BIOSTATISTICS AND CLINICAL BIOINFORMATICS"

MASTER THESIS

Perform a meta-analysis of CRTs for tacrolimus in atopic dermatitis published from 2005 to 2018.

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ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΕΡΓΑΣΤΗΡΙΟ ΒΙΟΜΑΘΗΜΑΤΙΚΩΝ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
"ΜΕΘΟΔΟΛΟΓΙΑ ΒΙΟΪΑΤΡΙΚΗΣ ΈΡΕΥΝΑΣ, ΒΙΟΣΤΑΤΙΣΤΙΚΗ ΚΑΙ ΚΛΙΝΙΚΗ ΒΙΟΠΛΗΡΟΦΟΡΙΚΗ"

ΘΕΜΑ ΔΙΠΛΩΜΑΤΙΚΗΣ ΕΡΓΑΣΙΑΣ

Πραγματοποίηση μεταανάλυσης για την φαρμακευτική ουσία τακρόλιμους σε ασθενείς με ατοπική δερματίτιδα, σε κλινικές μελέτες οι οποίες δημοσιεύτικαν το διάστημα 2005 μέχρι 2018.

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Contents

Perform a meta-analysis of CRTs for tacrolimus in atopic dermatitis published from 2005 to 2018.

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Abstract

Background: Atopic dermatitis (AD) is a skin condition which is characterised by redness and pruritus, That condition effects the quality of life for the patients whom are suffering. There is not known the cause of the illness or the cure as well. The first line treatment for AD was corticosteroids but for chronic, physicians suggested topical calcineurin inhibitors, such as pimecrolimus and tacrolimus ointments, to avoid corticosteroids side effects.

Aim: In order to ensure that tacrolimus, the most common calcineurin inhibitor is more effective than any other treatment a meta-analysis was performed in clinical trials from 2005 to 2018 which were testing the tacrolimus efficacy versus other known treatments.

Methods: Literature research was performed in the following databases, Cochrane Central Register of Controlled Trials (CENTRAL), LILACS Global Resource of Eczema Trials and Pub Med databases, in order to detect related papers. Odd ratio and forest plot were calculated to present the results. Egger test was performed to identify publication bias.

Results: Tacrolimus ointment is more effective, in contents of 0.1% and 0.03%, compared with other topical treatments in adults and adolescents.

Conclusion: According to the meta-analysis performed, there is statistically significant difference for most of the studies which include both adults and paediatric patients with AD, with tacrolimus being more effective than other treatments in any concentration.

Keywords: atopic dermatitis, meta-analysis, tacrolimus

Πραγματοποίηση μεταανάλυσης για την φαρμακευτική ουσία τακρόλιμους σε ασθενείς με ατοπική δερματίτιδα, σε κλινικές μελέτες οι οποίες δημοσιεύτικαν το διάστημα 2005 μέχρι 2018.

Πανεπιστημιο Θεσσαλιας Σχολη Επιστημων Υγειας, Εργαστήριο Βιομαθηματικών

Περίληψη

Ιστορικό: Η ατοπική δερματίτιδα (ΑΔ) είναι μια κατάσταση του δέρματος που χαρακτηρίζεται από ερυθρότητα και κνησμό, η οποία επηρεάζει την ποιότητα ζωής των ασθενών που υποφέρουν. Δεν είναι γνωστό ποιο είναι το αίτιο αυτής της ασθένειας και δεν υπάρχει γνωστή θεραπεία. Η θεραπεία πρώτης γραμμής για την ΑΔ είναι τα κορτικοστεροειδή, αλλά για χρόνια χρήση, οι γιατροί προτείνουν τοπικούς αναστολείς καλσινευρίνης, όπως αλοιφές πιμεκρόλιμους και τακρόλιμους, για την αποφυγή των παρενεργειών των κορτικοστεροειδών.

Στόχος: Προκειμένου να διασφαλιστεί ότι η τακρόλιμους, ο πιο κοινός αναστολέας καλσινευρίνης, είναι πιο αποτελεσματικός από οποιαδήποτε άλλη θεραπεία, πραγματοποιήθηκε μετα-ανάλυση στις κλινικές δοκιμές από το 2005 έως το 2018, οι οποίες δοκιμάζουν την αποτελεσματικότητα της τακρόλιμους έναντι άλλων γνωστών θεραπειών.

Μέθοδοι: Οι μέθοδοι που χρησιμοποιήθηκαν ήταν η βιβλιογραφική έρευνα στις Cochrane Central Register of Controlled Trials (CENTRAL), LILACS Global Resource of Eczema Trials and Pub Med βάσεις δεδομένων, ανίχνευση σχετικών δημοσιευμένων εγγράφων και ανάλυση του συνόλου των επιστημονικών δημοσιεύσεων για την ανίχνευση της συνολικής αναλογίας πιθανότητας και η δημιουργία ενός forest plot για την παρουσίαση των αποτελεσμάτων.

Αποτελέσματα: Το σκεύασμα τακρόλιμους σε αλοιφή είναι πιο αποτελεσματικό σε οποιαδήποτε περιεκτικότητα σε σύγκριση με άλλες θεραπείες σε ενήλικες και εφήβους.

Συμπέρασμα: Από την πραγματοποιηθείσα μετα-ανάλυση προκύπτει ότι οι περισσότερες από τις μελέτες που περιλαμβάνουν τόσο ενήλικες όσο και παιδιατρικούς ασθενείς με ΑΔ έχουν ως αποτέλεσμα ότι η τακρόλιμους είναι πιο αποτελεσματική από άλλες θεραπείες σε οποιαδήποτε συγκέντρωση.

Λέξεις κλειδιά: ατοπική δερματίτιδα, μετα-ανάλυση, τακρόλιμους

1. INTRODUTION

1.1 Atopic dermatitis

Atopic dermatitis is a chronic, relapsing, skin disease which effects the patients' quality of life and productivity. It appears with skin inflammation and it is characterised by pruritus, swollen, red and cracked skin. As a condition tends to flare periodically and may be accompanied by asthma or hay fever. Patients with atopic dermatitis often appear food allergies (Caproni; et al. 2007).

There is not known cause of atopic dermatitis, although genetic factors, immune system dysfunction and environmental exposures believed to be involved. People leaving in urban areas and dry climates appear to have higher rate to appear the symptoms of the disease. Stress can influence the symptoms but it does not consider as a cause (A.Kirschbaum; et al.2002).

There is not deference in the rate that both sexes are affected. A higher ratio occur for younger children to disease than adults, although there is a persist into adulthood in a 30% estimation of the cases.

Treatment involves a good management of anything conjuncts with flares of the disease and attempt to extend the remission periods between the flares. Management involves avoid skin contact with irritant factors such as wool clothing or perfumes, daily baths and moisturising cream application, usage of topical steroids and medication for pruritus as a first line treatment and long term usage of calcineurin inhibitors (Kirsner; et al. 2010)

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1.2 Pharmaceutical substances used in studies

1.2.1 Tacrolimus ointment 0.1% & 0.03%

Tacrolimus ointment is a drug for external use for truck and limps. It is working by suppresses the immune system and the inflammation by inhibiting the enzyme calcineurin which is crucial for the multiplication of T-cells, cells that are required for activation of the immune system. Tacrolimus was discovered in 1987 and tacrolimus ointment was approved for the treatment of atopic dermatitis in December 2000 (Paller; et al. 2005).

1.2.2 Pimecrolimus cream 1%

Pimecrolimus is an immunomodulating agent of the calcineurin inhibitor class used in the treatment of atopic dermatitis. Pimecrolimus, like tacrolimus, belongs to the ascomycin class of macrolactam immunosuppressives, acting by the inhibition of T-

cell activation by the calcineurin pathway and inhibition of the release of numerous inflammatory cytokines. Pimecrolimus has a similar mode of action to that of tacrolimus (Fleischr; et al.2007)

1.2.3 Methylprednisolone aceponate ointment 0.1%

Methylprednisolone aceponate is a glucocorticosteroid used to suppress the immune system and decrease inflammation. This drug is working by crossing cell membranes and bind with high affinity to specific cytoplasmic receptors, modifying transcription and protein synthesis. By this mechanism, Methylprednisolone aceponate can inhibit leukocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses.

Long-term use of methylprednisolone aceponate, as with all corticosteroids, can be associated with numerous of side effects as for instance swelling of face, weight gain, osteoporosis (Bieber; et al. 2007)

1.2.4 Hydrocortisone butyrate ointment 0.1%

Hydrocortisone butyrate is a corticosteroid belongs to the class of steroid hormones that are produced in the adrenal cortex of vertebrates. This medication is used to treat a variety of skin conditions as for example eczema, atopic dermatitis and allergies. Hydrocortisone butyrate stimulate the lipocortin-1 escaping to the extracellular space, where it binds to the leukocyte membrane receptors and inhibits various inflammatory events such as epithelial adhesion, chemotaxis, phagocytosis, and the release of various inflammatory mediators from neutrophils, macrophages and mastocytes. Additionally the immune system is suppressed by hydrocortisone butyrate due to a decrease in the function of the lymphatic system, a reduction in immunoglobulin and complement concentrations, the precipitation of lymphocytopenia, and interference with antigen-antibody binding (Reitamo; et al.2005)

1.3 AIM OF THE STUDY

The purpose of the study was to perform a meta-analysis in the clinical research trials between 2005 and 2018 which are testing tacrolimus, the most common calcineurin inhibitor versus other treatments sush as Hydrocortisone butyrate ointment 0.1%, Methylprednisolone aceponate ointment 0.1% and Pimecrolimus cream 1% in order to identify that tacrolimusis more effective than any other treatment.

2 MATERIALS AND METHODS

2.1 Literature search

All of the studies, published between July 2005 and 2018 were found after literature research in online databases in particular Cochrane Central Register of Controlled Trials (CENTRAL), LILACS Global Resource of Eczema Trials and Pub Med.

The research took place by searching for the following terms: atopic dermatitis, atopic eczema, tacrolimus, more effective, versus, compare, efficacy, pimecrolimus, glucocorticosteroids and corticosteroids.

2.2 Study selection

All of the articles were screened by title or abstract. If none of the exclusion criteria were satisfied, the article was candidate to be used in the present study.

Some studies used scoring systems with different diagnostic standards. Those studies were screened by reading the 'materials and methods' section of the papers. If there were general similarities with the majority of the studies, they were included.

The articles should compare tacrolimus ointment in any dilution, with other calcineurin inhibitors or corticosteroids, which are known to be effective in atopic dermatitis treatment.

All the studies published between 2005 and 2018.

2.3 Exclusion criteria

To insure the efficacy of the meta-analysis and minimise the heterogeneity of the study were created some cut off points, thus a number of studies was eliminated.

1. Studies that were comprised research in specific human race.
2. Studies that published before 2005.
3. Studies were the participants include infants.
4. Studies that were comparing tacrolimus with placebo were not included, since several studies had already proven the drug efficacy in this scenario.

2.4 Statistical formulas used

For the statistical analysis from the data given from the selected researches, extracted the number of participants of each group, tacrolimus group and control group, and the number of patients that treatment was successful for them. By transmitting these data to binary outcomes made possible to find the odds ratio of each treatment and the total odds ratio of all treatments combined.

$$OR = \left(\frac{\text{propability of succes in treated}}{\text{propability of succes in controls}} \right)$$

Then the Random Effect (RE) model used to synthesise the results so the overall estimation could be explored, because there is genuine diversity in the studies, as they are compare tacrolimus with different alternative treatments and the age groups are not the same in all studies (Michael; et al. 2010).

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$$\theta_p = \frac{\sum_{i=1}^{n} w_i \theta_i}{\sum_{i=1}^{n} w_i} = \frac{w_1\theta_1 + \ldots + w_n\theta_n}{w_1 + \ldots + w_n}$$

$$OR_p = e^{\theta_p} = e^{\ln(OR_p)} = 1.754$$

For RE model if the unite is not included in the odds ratio for 95% confidence interval the result is significant.

In order to examine the heterogeneity of the studies the following statistical formula has been used.

$$Q = \sum_{i=1}^{n} w_i \left(\theta_i - \theta_p\right)^2 = w_1 \left(\theta_1 - \theta_p\right)^2 + \ldots + w_n \left(\theta_n - \theta_p\right)^2$$

2.5 Data extraction

First name of the study	Year of publish	Mean age	Scoring system	Total cases	Total controls
Reitamo 2005	2005	32.5	Hanifin & Rajka Rajka &Langeland	972	485
Fleischer 2007	2007	39.65	Hanifin & Rajka *IGA	141	140
Paller 2005	2005	19.1	Hanifin & Rajka *IGA	528	532
Kirsner 2010	2010	17.8	Hanifin & Rajka *IGA	171	176
Bieber 2007	2007	7.65	*IGA	130	127

*Investigator Global Assessment for Atopic Dermatitis (IGA)

2.6 Statistical analysis

The main purpose of the present study was to juxtapose the potency of tacrolimus with other approved treatments for atopic dermatitis. After literature investigation were completed and data were extracted from the papers chosen, in order to estimate the overall difference between tacrolimus and the other known treatments, meta-analysis statistical formulas had to be done.

Odds ratio of each study used to perform a forest plot and present graphically the total result after categorising all data that had been extracted binomially.

Thus, prior to synthesis of the results from each individual research, the odds ratio specified as measure to be possible to calculate the differences. The random effects model (RE) has been used, as genuine diversity existed between the clinical trials.

The publication bias calculated with funnel plot, performed with IBM SPSS version 24 software and with Egger's test (Egger lee 2015)

All calculations made by using the excel formula provided by M.SC. course "Research Methodology In Biomedicine, Biostatistics And Clinical Bioinformatics" (Zintzaras 2010).

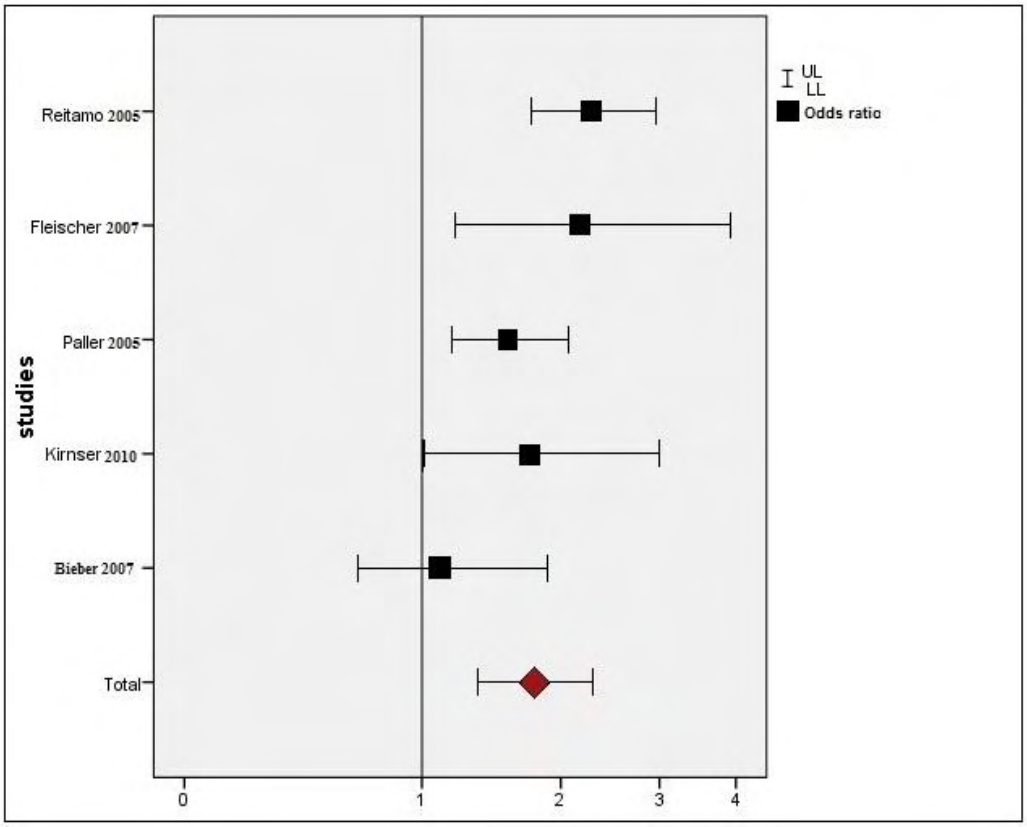
The results were graphical displayed by a forest plot made with the IBM SPSS version 24 software and by DistillerSR online tool.

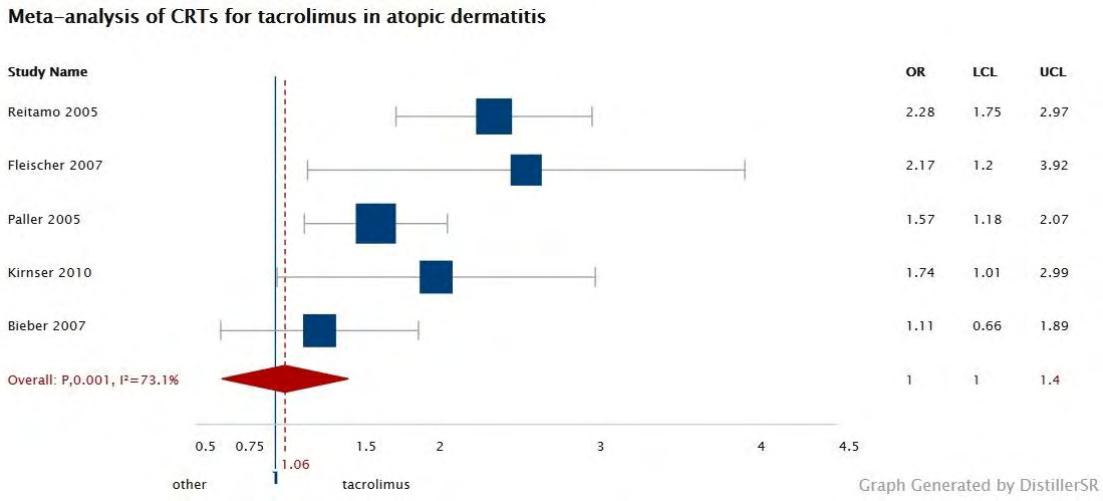
3 RESULTS

From a total of 2917 patients that were randomised in these five selected studies, where tacrolimus was compared with other topical treatments, the result was that there is statistical significant difference between tacrolimus and other treatments, with tacrolimus appear to be more effective.

Name of study	Odds ratio	Lower Limit	Upper Limit	θ=ln(OR)	√w	θ√w
Paller 2005	1.57	1.18	2.07	0.45	6.957	3.117
Fleischer 2007	2.17	1.20	3.92	0.77	3.306	2.557
Kirnser 2010	1.74	1.01	2.99	0.55	3.631	2.012
Bieber 2007	1.11	0.66	1.89	0.11	3.710	0.395
Reitamo 2005	2.28	1.75	2.97	0.82	7.385	6.077

In the following charts there is a summarise of the results in forest plots.





3.1 Results explanation

From the five studies selected, only two of them Reitamo et al 2005 and Bieber et al 2007,were compared tacrolimus versus ointments that contain steroids and the other studies (Kirnseret al 2010, Paller et al 2005 and Fleischer et al 2007) were compared tacrolimus versus pimecrolimus cream.

All the studies apart of Bieber et al 2007were included only adult patients or adult and pediatric patients, but Bieber et al 2007 was included children and adolescents.

The pooled estimation of the studies have as result that tacrolimus ointment has statistically significant better results that treatments compared with.

All the studies with odds ratio over 1.00 have as statistically significant result that tacrolimus is better than the opposite treatment. The lower limit of the confidence interval for the study Kirnser et al 2010 is touching axis of one, so the result ismarginally statistically significant.

The confidence interval for Bieber'sstudy2007 crossed the axis of one so the result is not statistically significant.

In forest plots in general the bigger the square of a study is, the weightier the study is, that shows, according to DistillerSR online tool, that Paller 2005 is the most weighted study and Fleischer 2007 the less weighted study.

The same studies have the smallest and biggest confidence interval vice versa.

3.2 Heterogeneity

By using, the statistical formula presented in chapter 'Statistical formulas used' a P-value of 0.057 found. That result confirm statistically significant heterogeneity, because if P-value < 0.10 significant heterogeneity exists. This result can be explained by theory, as the treatments compared with tacrolimus are not the same for all papers selected.

3.3 Publication bias

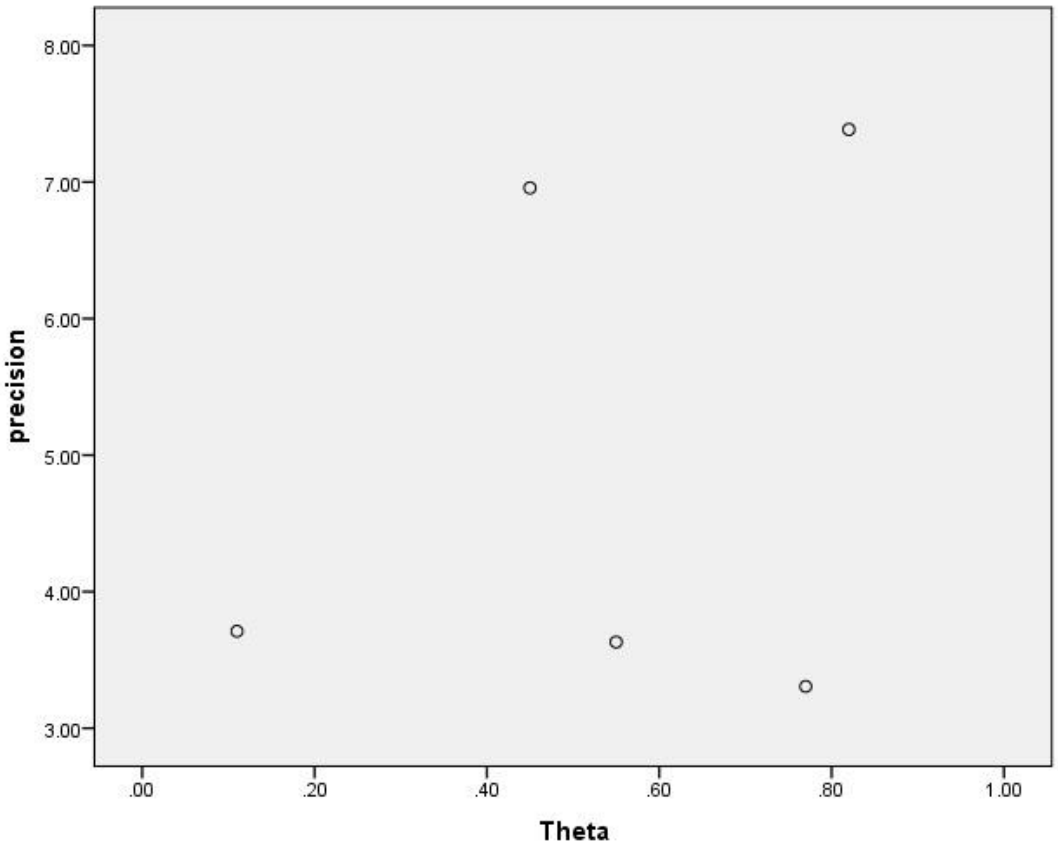
In order to accept the results a publication bias test should take place to ensure that not only papers with significant results were selected to be analysed for the meta-analysis.

Therefore the common used funnel plot had been used, because it is easier to be red, as the results appear graphically in a chart.

Hence the SPSS version 24 software had been used by adding the theta and the precision of each study and perform the graph.

$$\textbf{Theta} = \theta_i = \ln(OR_i)$$

$$\textbf{Precision} = 1/SE_i$$



The graph predisposes us for not publication bias, but because of the small number of the studies, it is not clear if publication bias exists.

For that reason an Egger's test performed next, to insure that publication bias does not exist.

To complete the Egger's test with SPSS version 24 software, $\theta i\sqrt{wi}$ and $\sqrt{wi}$ had been used.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.785[a]	.616	.488	1.48760

a. Predictors: (Constant), X

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10.659	1	10.659	4.817	.116[b]
	Residual	6.639	3	2.213		
	Total	17.298	4			

a. Dependent Variable: Y

b. Predictors: (Constant), X

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.257	1.978		-.636	.570
	X	.818	.373	.785	2.195	.116

Because P-value = 0.570 ≥ 0.05 it is safely proven that there is not publication bias.

4 Limitations

The limitations of the study that have to be mentioned are mainly two, the number of studies and the heterogeneity.

4.1.1 Number of studies

The number of the studies selected can be considered as small and that fact can lead in high heterogeneity.

4.1.2 Heterogeneity

The heterogeneity observed between the studies is high, and that is because of the small number of the studies and the differences between what studies are comparing.

The studies Reitamo 2005 and Bieber 2007 are comparing tacrolimus with ointments which contain steroids, the other tree studies compare tacrolimus with pimecrolimus.

The other heterogeneity limitation that is observed in the study is that Bieber 2007 study does include only children and adolescents patients counter to the other four studies.

A subgroup analysis can be proposed for further investigation.

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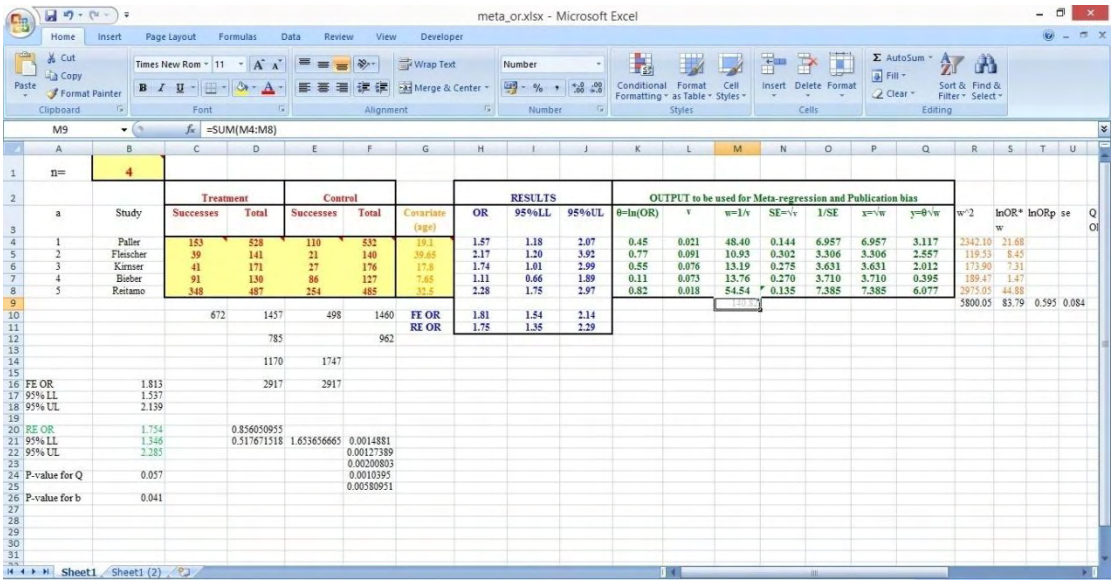
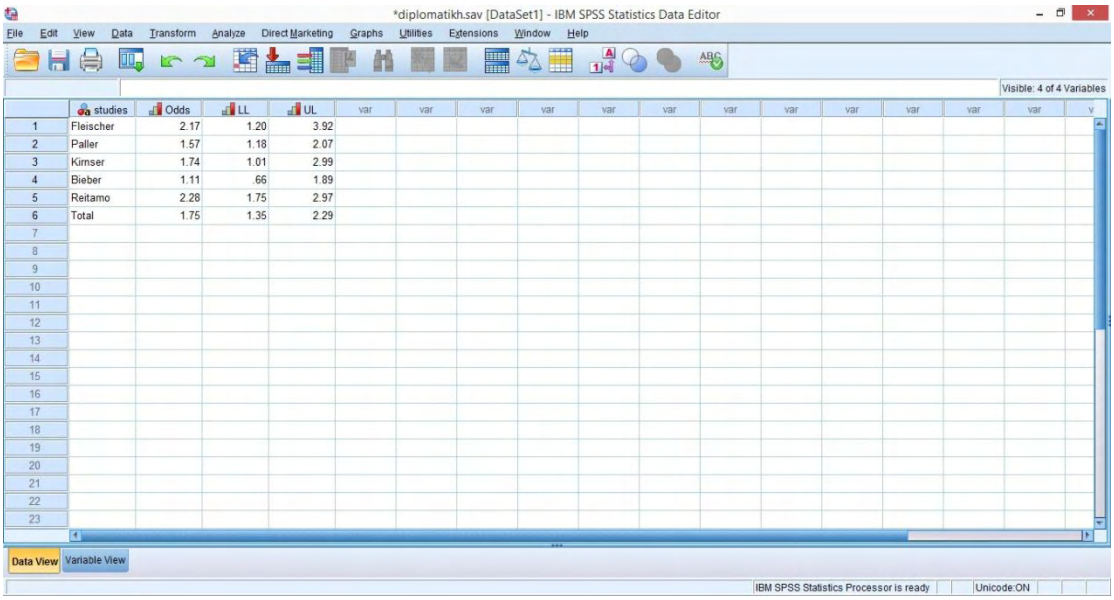
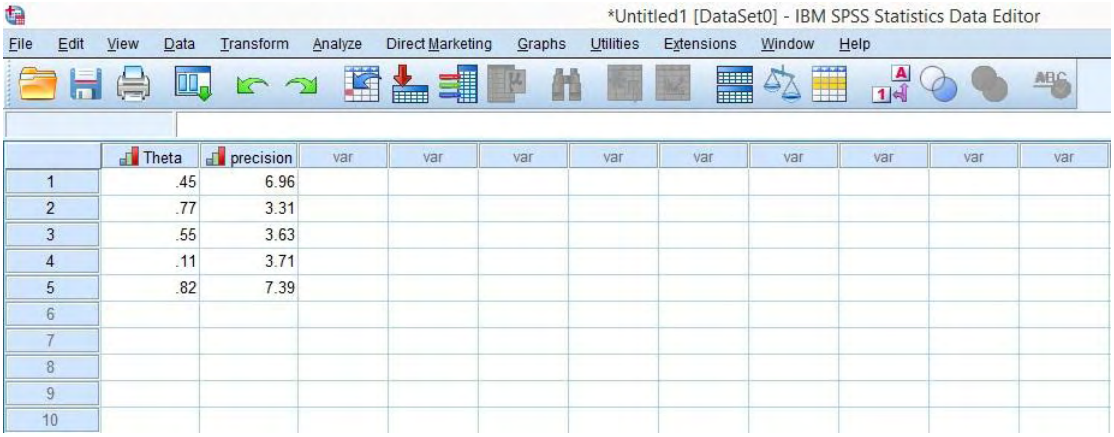
6 Appendix



Calculations with excel provided by M.SC. course "Research Methodology In Biomedicine, Biostatistics And Clinical Bioinformatics" (Zintzaras.2010).

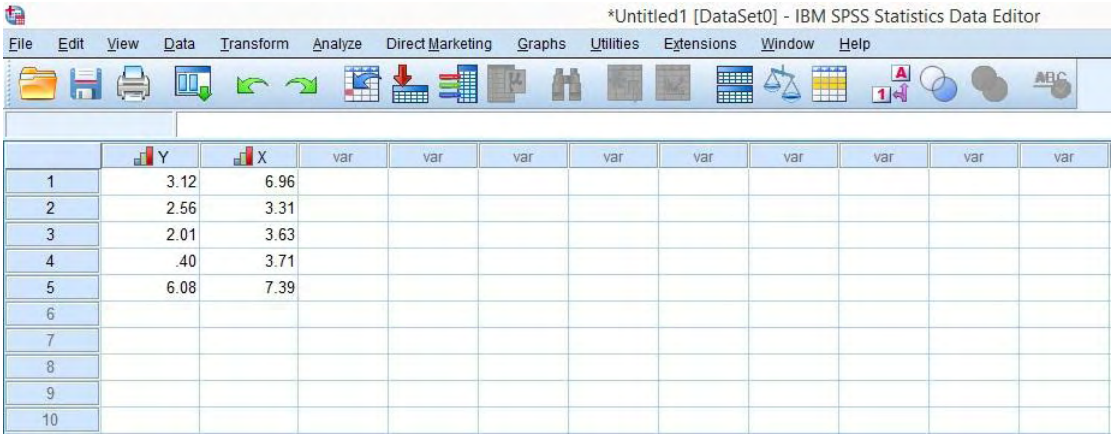


IBM SPSS version24 software used to built forest plot.



	Theta	precision	var	var	var	var	var	var	var	var	var
1	.45	6.96									
2	.77	3.31									
3	.55	3.63									
4	.11	3.71									
5	.82	7.39									
6											
7											
8											
9											
10											

IBM SPSS version24 software used to built funnel plot.



	Y	X	var	var	var	var	var	var	var	var	var
1	3.12	6.96									
2	2.56	3.31									
3	2.01	3.63									
4	.40	3.71									
5	6.08	7.39									
6											
7											
8											
9											
10											

IBM SPSS version24 software used to perform Egger’s test.